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Recent advances in metal carcinogenicity*

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Abstract: The carcinogenicity of nickel, chromium, arsenic, cobalt, and cadmium compounds has long been recognized. Nevertheless, the mechanisms involved in tumor formation are not well understood. The carcinogenic potential depends on metal species; major determinants are oxidation state and solubility. Two modes of action seem to be predominant: the induction of oxidative DNA damage and the interaction with DNA repair processes, leading to an enhancement of genotoxicity in combination with a variety of DNA-damaging agents. Nucleotide excision repair (NER) is inhibited at low, non-cytotoxic concentrations of nickel(II), cadmium(II), cobalt(II), and arsenic(III); the repair of oxidative DNA base modifications is disturbed by nickel(II) and cadmium(II). One reason for repair inhibition appears to be the displacement of zinc(II) and magnesium(II). Potentially sensitive targets are so-called zinc finger structures present in several DNA repair enzymes such as the mammalian XPA protein and the bacterial formamidopyrimidine-DNA glycosylase (Fpg protein); detailed studies revealed that each zinc finger protein exerts unique sensitivities toward toxic metal ions. Taken together, toxic metal ions may lower the genetic stability by inducing oxidative DNA damage and by decreasing the repair capacity towards DNA lesions induced by endogenous and exogenous mutagens, which may in turn increase the risk of tumor formation.

CARCINOGENIC METAL COMPOUNDS: OCCURRENCE, CHEMICAL SPECIATION, AND INTERFERENCE WITH CELLULAR FUNCTION RELATED TO CARCINOGENICITY

Metal compounds are part of the earth crust and thus ubiquitously distributed in the environment. Yet, combustion and industrial use contribute significantly to human exposure at workplaces and in the general environment. Some metals, including chromium, nickel, arsenic, cadmium, and cobalt, have long been recognized as human and/or animal carcinogens [1–4]. Their carcinogenic potentials depend largely on factors like oxidation state and solubility. Thus, exposure to chromium(VI) is strongly associated to human lung cancer, while chromium(III) is largely inactive. This discrepancy is related to differences in bioavailability; while chromium(III) is unable to cross the cell membrane, chromium(VI) is readily taken up by anion transporter followed by intracellular reduction to chromium(III) (reviewed in ref. 5).

The impact of solubility is most evident for nickel compounds. While particulate nickel compounds with intermediate water solubility (like nickel subsulfide) are strong carcinogens, soluble nickel(II) salts exert considerably weaker effects. This difference could be attributed to differences in bioavailability. While water-soluble nickel salts are taken up only slowly by cells, particulate nickel compounds are phagocytosed and gradually dissolved in lysosomes because of the low pH, yielding high concentrations of nickel ions in the nucleus [6]. Nevertheless, the underlying molecular mechanisms of metal carcinogenicity are not well understood, especially since they are not mutagenic in bacterial test systems and only weakly mutagenic in cultured mammalian cells. Recent studies have identified levels of interaction which may be relevant for the carcinogenic process. They include the induction of oxidative DNA damage [7], the interference with diverse DNA repair systems [8], as well as changes in the expression of certain oncogenes or tumor suppressor genes by interference with signal transduction

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processes [9] or changes in DNA methylation patterns [10,11]. The first two aspects will be discussed in more detail in this paper.

OXIDATIVE DNA DAMAGE

Reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) and superoxide anions ($O_2^{\cdot-}$) are continuously generated during oxygen metabolism. While neither molecule reacts directly with DNA, they are converted into the highly reactive hydroxyl radical via Fenton- and Haber-Weiss type reactions in the presence of redox-active transition metals. One frequently discussed mechanism in metal-induced genotoxicity is the formation of oxidative DNA damage. This is best established for chromium(VI) compounds. After uptake, chromium(VI) undergoes intracellular reduction to the stable chromium(III) species. In the course of reduction, reactive intermediates (ROS, sulfur-centered radicals, pentavalent and tetravalent chromium species) are generated, and different forms of structural DNA damage, including 8-oxo-guanine as an indicator of oxidative DNA damage, are induced (for recent review see ref. 12). Further compounds found to damage DNA in the presence of H_2O_2 *in vitro* are iron(II)/iron(III), nickel(II), and cobalt(II). Oxidative DNA damage might occur also via the inactivation of cellular defense systems, as has been postulated in the case of cadmium(II) (for recent review, see ref. 7).

Whether or not the induction of oxidative DNA damage is relevant for realistic exposure conditions cannot readily be answered from subcellular test systems. The most reactive oxygen species like hydroxyl radicals are rather short-lived, and intact cells have defense systems to detoxify oxygen radicals. Therefore, the potential relevance of oxidative damage for living cells depends on uptake, complex ligands, intracellular distribution, and reductants, all of which affect the concentrations of metal ions available for redox reactions close to critical targets like the DNA.

Interestingly, amino acid-binding motifs for Ni(II) and Cu(II) in core histones H3 and H2A, as well as in human protamines replacing histones in sperm DNA, have been identified and the formation of 8-oxo-guanine in the presence of H_2O_2 has been demonstrated in the latter system [13,14]. Nevertheless, when investigating the induction of DNA strand breaks and oxidative DNA base modifications in HeLa cells, we observed that both nickel(II) and cadmium(II) induce DNA strand breaks in a dose-dependent manner, starting at non-cytotoxic concentrations. In contrast, the induction of specific oxidative DNA base modifications was restricted to high, cytotoxic concentrations of nickel(II), and no increase was observed after treatment with cadmium(II) [15]. These observations seem to contradict the occurrence of oxidative DNA base damage in cobalt(II) and nickel(II)-treated animals [7]; however, these lesions may not be induced directly, but rather be due to an accumulation of unrepaired endogenous DNA lesions in the presence of metal ions (see below).

INTERACTIONS WITH DNA REPAIR SYSTEMS

In spite of the lack of mutagenicity of metal compounds in bacterial test systems, arsenic(III), copper(II), nickel(II), cadmium(II), and manganese(II) increased the mutation frequency when combined with UV-light and/or alkylating agents. This suggests that an inhibition of DNA repair processes may be one predominant mechanism in metal-induced genotoxicity. In addition, most carcinogenic metal compounds have been shown to increase the cytotoxicity, mutagenicity, and clastogenicity in mammalian cells when combined with different types of DNA-damaging agents (for review, see ref. 16). In the case of arsenic(III), nickel(II), cadmium(II), and cobalt(II), these effects were found in a close relationship to interactions with DNA repair processes.

Nucleotide excision repair

Nucleotide excision repair (NER) is the major repair system involved in the removal of DNA damage induced by UV radiation, environmental and food mutagens such as benzo(a)pyrene as well as DNA

lesions generated by some cytostatic drugs like cis-diaminedichloroplatinum(II) (cisplatin). The repair process involves at least 30 different proteins and enzymes in mammalian cells, including those missing or defective in patients suffering from the DNA repair disorder xeroderma pigmentosum (XP) complementation groups A through G. It can be roughly subdivided into three different steps, namely the incision at both sides of the lesion, the repair polymerization leading to the displacement of the damaged oligonucleotide, and finally the ligation of the repair patch (for recent review, see ref. 17).

By investigating the effect of metal compounds on the removal of DNA lesions induced by UV radiation and by applying inhibitors of distinct steps of this repair pathway, compounds of nickel(II), cobalt(II), cadmium(II) and arsenic(III) have been shown to inhibit nucleotide excision repair. For all metal compounds investigated, the incision step was affected at the lowest concentrations [18–20]. However, cobalt(II) inhibited additionally the polymerization and arsenic(III), as well as nickel(II), the ligation step of the repair process, although at higher concentrations [18,20,21].

One important prerequisite for the initiation of repair events is the recognition of the DNA lesions, and some proteins involved in DNA damage recognition have been identified recently. Recent findings suggest that binding of XPC/HHR23B complex is the initial, damage-recognizing step in NER. XPA, which also exerts high affinity for damaged DNA may verify the damage and organize the other repair proteins around the lesion. Finally, even though not essential for DNA repair in vitro, XPE is thought to serve as an accessory factor in damage recognition [17].

With respect to those metals inhibiting the incision step of nucleotide excision repair, we investigated whether they disturb the actual incision reaction or interfere with DNA damage recognition after treatment of intact cells with low, biologically relevant concentrations of the metal compounds.

DNA damage recognition requires the preferential binding of one or more proteins to damaged DNA as compared to undamaged DNA, which has been studied by gel mobility shift assay applying an UVC-irradiated synthetic oligonucleotide and nuclear extracts prepared from control or metal-treated HeLa cells. A diminished protein binding was observed with nuclear extracts derived from HeLa cells treated with 50 μM nickel(II) and higher; however, the binding was largely restored when increasing the concentration of magnesium(II) in the gel-shift reaction, indicating the competition of both metal ions for protein binding sites. In the case of cadmium(II), a dose-dependent reduction of DNA-protein interactions was seen at concentrations of 0.5 μM and higher. In contrast to the results obtained with nickel(II), an increase in magnesium(II) had no impact on the observed inhibition; however, the addition of 100 μM zinc(II) to the binding reaction led to a largely restored binding behavior at all cadmium-concentrations applied [22].

Base excision repair

In addition to bulky lesions removed by NER, some forms of modified DNA bases are repaired by base excision repair (BER). According to current models, BER is started by removal of the modified base by a specific N-glycosylase, generating an AP site, which is subsequently incised at its 5' side by an AP endonuclease. After excision of the 5' terminal dRP, the single nucleotide gap is filled by DNA polymerase β and sealed by ligase. Alternatively, AP sites are further processed involving proliferating cell nuclear antigen and DNA polymerase δ for the excision and DNA synthesis reaction [23]. DNA glycosylases act specifically on one or few substrates, and DNA base excision repair is mainly responsible for the removal of different types of endogenous DNA damage, including some alkylated DNA bases and oxidative DNA base modifications arising as a consequence of oxygen consumption.

Concerning the interactions of toxic metal compounds with BER, arsenite inhibits the ligation step during the repair of N-methyl-N-nitrosourea-induced DNA damage [24]. With respect to oxidative DNA damage, the effect of nickel(II) and cadmium(II) on the repair of oxidative DNA base modifications induced by visible light was investigated in HeLa cells. Current evidence suggests that the prevailing oxidative DNA base modification generated by this treatment is 7,8-dihydro-8-oxoguanine (8-hydroxyguanine), a premutagenic DNA base modification, and to a lesser extent DNA strand breaks,

presumably due to the reaction of singlet oxygen or excited intracellular photosensitizer molecules with DNA [25–27]. Both cadmium(II) and nickel(II) inhibited the repair of the induced DNA damage at low, non-cytotoxic concentrations. In the case of nickel(II), the closure of DNA strand breaks and the removal of bacterial Fpg protein-sensitive sites were inhibited at concentrations of 100 μM and 50 μM, respectively. While cadmium(II) had no effect on the repair of DNA strand breaks, the repair of the oxidative DNA base modifications was impaired at 0.5 μM and higher [15]. Therefore, even though oxidative DNA base modifications are not induced by both metal compounds at biologically relevant concentrations, their extent may be enhanced indirectly by nickel(II) and cadmium(II). Since oxidative DNA damage is induced continuously in living cells due to oxygen metabolism, an impaired repair of these lesions may result in an increased background frequency of the respective DNA lesions.

Repair of O^6-methylguanine

The mutagenicity and carcinogenicity of N-nitroso compounds is thought to be a consequence of DNA alkylation occurring to different extents and at different positions of DNA bases, yielding predominantly 7-alkylguanine, 3-alkyladenine, O^6-alkylguanine and O^4-alkylthymine [28]. O^6-methylguanine, in particular, and O^4-methylthymine are premutagenic and precarcinogenic lesions, and the tumor frequency in different organs depends, at least partly, on the efficiency of their removal. As opposed to nucleotide and base excision repair, the elimination of both lesions depends on a single repair protein, namely O^6-methylguanine-DNA-methyl transferase (MGMT). During the repair reaction, the methyl group is transferred to MGMT itself, which becomes irreversibly inactivated [29]. Transgenic cells and animals overexpressing MGMT are highly protected from genotoxicity and carcinogenicity induced by alkylating agents [30,31]. When quantifying the removal of N7-methylguanine and O^6-methylguanine in MGMT-proficient Chinese hamster ovary (CHO) cells in the presence and absence of nickel(II), we observed a pronounced inhibition of removal of O^6-methylguanine, while no effect was seen in case of N7-methylguanine [32].

Potential mechanisms of repair inhibition

Even though the inhibition of DNA repair processes appears to be one common feature of some carcinogenic metal compounds, the steps affected and thus the reasons for repair inhibition seem to be quite different. One mechanism of repair inhibition appears to be the displacement of essential metal ions such as zinc(II) in case of cadmium(II) and magnesium(II) in case of nickel(II) and cobalt(II). Since metal ions are cofactors in many cellular processes and all repair inhibitions have been observed at low, non-cytotoxic concentrations of the respective metal compounds, this raises the question why DNA repair systems are sensitive toward toxic metal ions.

Some toxic metal ions exert high affinities toward SH groups; therefore, potential targets are so-called zinc finger proteins. They comprise a family of proteins where zinc is complexed through four invariant cysteine and/or histidine residues forming a zinc finger domain, which is mostly involved in DNA binding, but also in protein-protein-interactions [33]. Even though most zinc finger structures have been described as DNA-binding motifs in transcription factors, they have also been identified in several DNA repair enzymes. They include the mammalian XPA protein essential for DNA damage recognition during NER as well as the bacterial Fpg protein involved in the removal of oxidative DNA base modifications. Thus, we applied both proteins as models to analyze their response to toxic metal ions. The Fpg protein was inhibited by cadmium(II), copper(II), and mercury(II), whereas cobalt(II), arsenic(III), lead(II) and nickel(II) had no effect. Nevertheless, besides cadmium(II) and copper(II), both nickel(II) and cobalt(II) inhibited DNA binding of XPA, while mercury(II), lead(II), and arsenic(III) were ineffective (ref 34). Thus, zinc finger structures may be sensitive targets for toxic metal com-

pounds, but each zinc finger protein exerts its unique sensitivities. The data add further evidence that there is no single mechanism accounting for metal-induced repair inhibitions.

CONCLUSIONS AND PERSPECTIVES

Taken together, toxic metal ions may damage the genetic information by different mechanisms. How do these observations contribute to understanding metal-induced carcinogenicity? As shown in Fig. 1, tumor development is a multistep process, resulting in mutations in several oncogenes and/or tumor suppressor genes, which can frequently be detected in tumor tissue. According to a current model, in most cases these mutations are preceded by mutations in genes related to DNA stability, such as those involved in DNA repair, DNA replication, or chromosome segregation. Mutations in such DNA stability genes, in turn, increase the probability of mutation fixation as response to DNA damage. If these mutations occur in oncogenes and/or tumor suppressor genes, they may provide advantages in cell growth and thus lead to tumor development.

This model also points out the importance of DNA repair processes. DNA is continuously damaged not only by environmental agents such as UV radiation, but also by endogenous processes, leading to several thousand lesions per cell and day [36]. The efficient repair of these lesions is thus an important prerequisite to maintain DNA integrity. If repair is not functioning, cells may accumulate DNA

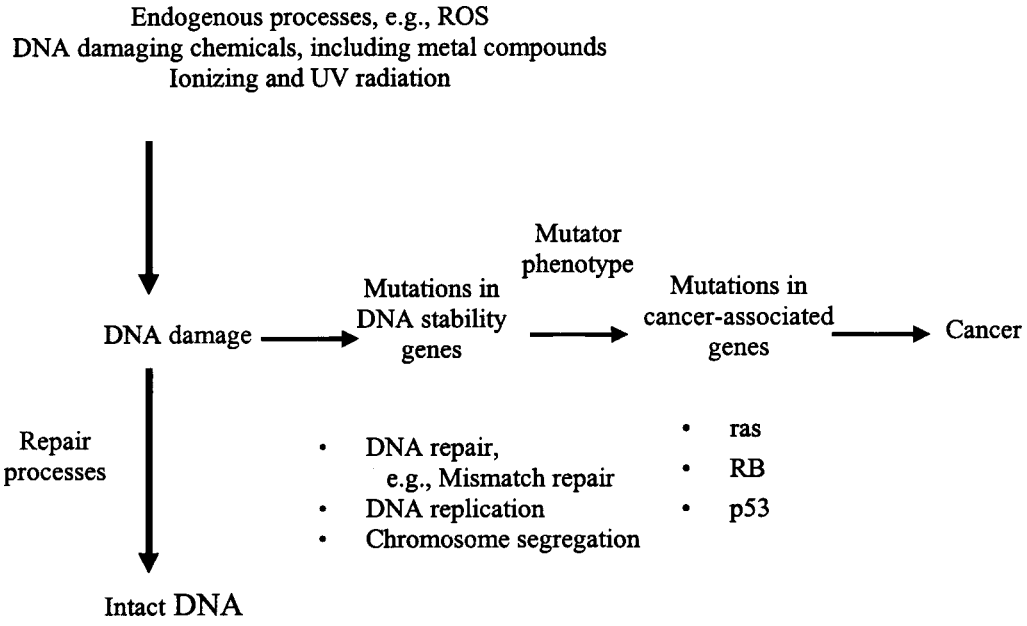

Fig. 1 Current model explaining the occurrence of multiple mutations in cancer (modified from ref. 35).

damage, thereby generating a mutator phenotype. This model is supported by high tumor incidences in patients suffering from rare nucleotide excision repair deficiency syndromes like xeroderma pigmentosum [17] or defects in mismatch repair involved in the correction of DNA replication errors, which have been associated with increased susceptibility to hereditary nonpolyposis colon cancer (HNPCC) [37].

Taken together, there are different levels of interaction by toxic metal compounds with potential impact on their carcinogenic potential (Fig. 2). Nevertheless, since metal ions have many potential cellular binding sites, thus intracellular targets, the concentrations required to exert the respective effect may be critical. From our experiments in HeLa cells, it appears that the induction of oxidative DNA

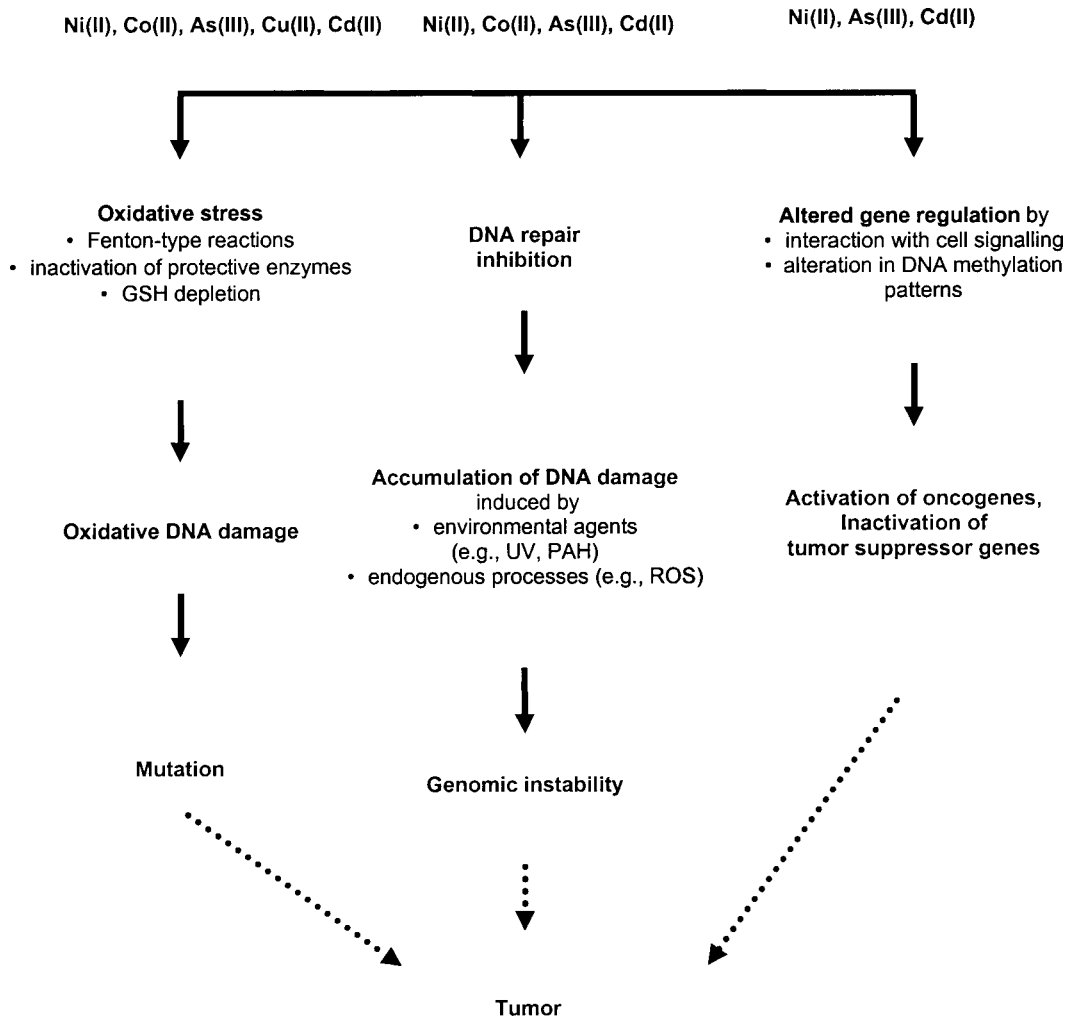

Fig. 2 Proposed mechanisms for metal-induced carcinogenesis. For further details see text.

base modifications by soluble nickel and cadmium compounds are restricted to high concentrations [15]. However, the situation *in vivo* may be different. For example, phagocytosis of particulate nickel compounds may lead to locally high concentrations of nickel ions in the nucleus. In contrast, the repair inhibitions described above have been observed at low concentrations, in most cases more than ten-fold below the cytotoxic level. Thus, under environmental and occupational exposure conditions repair inhibition may contribute significantly to metal-induced carcinogenicity.

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